#16 Declaration 7/5/01

THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Arntzen et al.

Application No.: 09/129,298 Group Art Unit: 1638

Filed: August 5, 1998 Examiner: O. Zaghmout

For: THE USE OF MIXED OLIGONUCLEOTIDES TO EFFECT LOCALIZED CHANGES IN PLANTS

Attorney Docket No.: 7991-023-999

DECLARATION OF DR. RICHARD A. METZ UNDER 37 C.F.R. § 1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, RICHARD A. METZ, do declare and state that:

1. I am a resident of the United States, residing at 37 Winthrop Road, Lawrenceville, New Jersey, 08648.

2. I presently hold the position of Executive Director of Product of Development, Human Healthcare Division at ValiGen (US), Inc., the assignee of the present application, which position I have held since November 1998. I held the positions of Director of Microbial Targeting and Bioanalytical Research at Kimeragen, Inc., the predecessor corporation of ValiGen (US), Inc., from February 1998 to November 1998; Director of Bioanalytical Research at Kimeragen, Inc. from January 1997 to February 1998; Director of Molecular Biology from April 1996 to January 1997 and Senior Scientist from September 1994 to April 1996 at Quality Biotech, Inc., Camden, New Jersey; post-doctoral research scientist at Bristol-Myers Squibb, Princeton, New Jersey from August 1991 to August 1994; post-doctoral fellow in the Departments of Pathology and Biochemistry, New York University Medical Center from February 1991 to August 1991; and Research Assistant at

HBF Labs, Newark, New Jersey from January 1984 to July 1984. Attached as Exhibit A is a copy of my *Curriculum vitae*.

3. I received the degree of Doctor of Philosophy from New York University Medical Center, New York, New York in 1991; a Masters of Science from Weizmann Institute of Science, Rehovot, Israel in 1983; and a Bachelors of Science from Purdue University, West Lafayette, Indiana in 1980.

4. I have read and am familiar with the specification of the above-identified application. I have been informed that claims of the above-identified patent application relating to methods of making a localized mutation causing a desired trait in a target gene in a plant cell comprising adhering to a particle a recombinagenic oligonucleobase, introducing the particle into a cell of a population of plant cells and identifying a cell of the population having a mutation, or comprising perforating the cell walls of a population of plant cells, introducing a recombinagenic oligonucleobase and identifying a cell of the population having a mutation, are subject to a rejection based on obviousness because it is alleged that the teachings of U.S. Patent Nos. 5,565,350; 5,731,181; and 5,204,253 render obvious the claimed methods to one skilled in the art.

5. I am a scientific investigator and manager of technical research and development. My professional research interests have focused upon a number of areas including gene therapy, homologous recombination, DNA repair and gene targeting in microbial systems. My work has focused on the molecular biology of human and microbial systems for the last twenty years. I have been involved in gene therapy, site-directed mutagenesis, and more recently commercial applications of recombinagenic nucleobases.

6. My research experience also includes the use of biolistics technology, the technology described in U.S. Patent No. 5,204,253. More than one thousand uses of recombinagenic oligonucleobases and more than fifty uses of biolistics technology in microbial systems have been performed either by me personally or under my supervision and control. As a consequence of my own experience and knowledge of the scientific literature concerning molecular biotechnology, I am very familiar with the technical capabilities of

those who routinely perform research in the fields of site-directed mutagenesis, gene targeting and the use of recombinagenic oligonucleobases.

7. The biolistics technology taught by U.S. Patent No. 5,204,253 and the present specification is a technology used, *inter alia*, to transfer nucleic acid molecules into a cell. The technology requires that the nucleic acid molecules be precipitated onto microparticle projectiles, usually a suspension of gold particles one micron in diameter, in a harsh solution of salts, *e.g.*, calcium chloride, and positively charged proteins, *e.g.*, spermadine. The nucleic acid-coated particles are then literally "shot" into cells.

8. In complete contrast, the recombinagenic oligonucleobases of the present invention are radically different in size and structure than the nucleic acid molecules that were typically employed and known to work in biolistics technology applications at the time the present invention was made. The recombinagenic oligonucleobases of the present invention are small single-chained molecules with short regions of secondary structure. This secondary structure is fragile because of its limited length but is critical for maintaining functionality of the molecule. Based on experiments performed in the laboratories of ValiGen (US), Inc., it is known that the measurable activity of the recombinagenic oligonucleobases is lost if the recombinagenic oligonucleobases are repeatedly frozen and thawed in an aqueous solution. The oligonucleobases also lose activity over time when maintained at 4°C in an aqueous solution, and under some circumstances losing all measurable activity after being stored for a couple of weeks. It is believed that the loss in activity is due to the loss of required secondary structure since renaturation of the oligonucleobase molecule results in a restoration of activity. The loss of secondary structure is not a problem with most transformation systems since recombinagenic oligonucleobases can be renatured before use, even after following exposure to conditions that would destroy any secondary structure or favor inactive secondary structure or concatamer formation.

9. However, after adherence of the recombinagenic oligonucleobases to a particle in accordance with biolistics technology described in U.S. Patent No. 5,204,253 and in the present specification, the option of renaturing the oligonucleobase molecules is not available

since that might result in stripping the oligonucleobase molecules from the particle. Therefore, based on the foregoing, it is my opinion, and I believe that a scientist knowledgeable in the field of chimeraplasty and molecular biology of human and microbial systems would also hold the opinion, that the cited prior art references do not provide the required reasonable expectation of success in achieving the claimed methods because it could not have been reasonably predicted that the short regions of secondary structure of the recombinagenic oligonucleobases would survive the harsh conditions under which the oligonucleobases are precipitated onto the gold particle, *i.e.*, it could not be reasonably predicted that the secondary structure required for activity of the oligonucleobase molecules would be maintained.

10. Based on my research experience and the foregoing discussion, I conclude, and I believe a scientist knowledgeable in the field of chimeraplasty and molecular biology of human and microbial systems would also conclude, that the teachings of U.S. Patent Nos. 5,565,350; 5,731,181; and 5,204,253, either alone or in combination, do not render obvious methods of making a localized mutation causing a desired trait in a target gene in a plant cell comprising adhering to a particle a recombinagenic oligonucleobase, introducing the particle into a cell of a population of plant cells and identifying a cell of the population having a mutation, or comprising perforating the cell walls of a population of plant cells, introducing a recombinagenic oligonucleobase and identifying a cell of the population having a mutation, because the prior art does not provide a reasonable expectation of success in achieving such methods since recombinagenic oligonucleobases are sufficiently different from the double-stranded and single-stranded nucleic acid molecules employed in prior art biolistics methods in that the molecules have regions of secondary structure which are required for activity. Accordingly, I conclude, and I believe a scientist knowledgeable in the field of molecular biology of human and microbial systems would also conclude, that the claimed methods of the present invention are nonobvious in view of the cited prior art.

11. I declare further that all statements made in this Declaration of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements and the like so made are punishable by fine or

imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the specification or any patent issuing thereon.

Dated: 6/12/01

Richard A. Metz, Ph.D.

Attachments:

Exhibit 1: *Curriculum vitae*